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OUR REF: 1160.003US1

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In re. Patent Application of: Roland Valdes, Jr. et al.
Serial No.: 09/503559
Filed: February 11, 2000
Examiner: Ulrike Winkler
Group Art Unit: 1648
Docket No.: 1160.003US1
Title: DIHYDROOUABAIN-LIKE FACTOR AND DIAGNOSTIC & THERAPEUTIC COMPOSITIONS AND METHODS

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There appears to be some confusion with regard to the interpretation of our experimental results surrounding the invention related to dihydro-OLF (Dh-OLF). The confusion may lie in the interpretation of the chromatographic mobility of certain compounds as they relate to the resolution of those compounds by chromatography and as to the use of the word *identical* in the original description.

First, we will address the apparent misunderstanding concerning the separation of the compounds in question as originally submitted and also reported in our publication in 2000 (Qazzaz et al., Endocrinology 2000;141(9):3200-3209). The Patent Office indicates an apparent discrepancy in the original submission, which indicated that *"the Dh-OLF isolated here-in showed an identical chromatographic retention to the standard component dho-B"*. It is important to note that the word *identical* is limited to the technology and conditions under which the measurements where performed.

In the above publication the inventors reported the presence of endogenous mammalian Dh-OLF (from bovine adrenal cortex, human serum and mouse Y-1 adrenocortical cell line) as detected using a combination of antibody raised against commercially available dihydrouabain (containing both isomers A & B) and HPLC chromatography. In these experiments, the inventors reported a "similar" average elution time (27.5 min) for Dh-OLF (mammalian-derived) to the one reported in their 1997 abstract (see below) for the commercial plant-derived isomer of dihydroouabain (dho-B). It is very important to note that in the 2000 article (Panel A of Figure 2) they measured only the absorbance of the dho-B and that the peak obtained is rather broad as originally reported in our 1997 abstract (see below). This "broadness" in the elution peak has to do with the fact that a very high concentration (micromolar quantities) is necessary in order to measure these kinds of compounds using absorbance. Large amounts of compounds injected into the HPLC affects chromatographic resolution between similar compounds. However, note that in Panel B and C of the same Figure 2 they measured both the absorbance and the immunoreactivity (by antibodies) to detect the mammalian Dh-OLF (which chromatographically migrated in the "same region" as did the plant-derived dho-B). This is important, because under those conditions the limited resolution of the HPLC procedure would not allow them to detect subtle differences in the elution time between the plant-derived dho-B and Dh-OLF. Thus, they concluded that one of the properties characterizing their Dh-OLF was an "identical" (implying very similar) chromatographic elution to plant–derived dho-B.

The question then arose from the Patent Office as to whether there was any evidence to indicate a structural difference existing between Dh-OLF (our invention) and the plant-derived compound dho-B, thus rendering assurances that Dh-OLF and dho-B are in fact structurally different substances. The limited resolution of the measuring techniques used in the 2000 publication would not be able to demonstrate subtle differences, thus the inventors demonstrated that these compound were in fact structurally distinct. By using techniques that provide higher chromatographic resolution (smaller quantities of these compounds, measuring them using antibodies, and mixing them together before chromatography), the inventors demonstrated definitively that Dh-OLF and dho-B, when mixed together and chromatographed using the conditions conducive to

greater chromatographic resolution, they do indeed separate. This is definitive proof that they are distinctly different in structure. It is important to understand that compounds with different structures can, when chromatographed by HPLC, migrate differently or similarly depending on the conditions of the experiment and that generally any claim of identical migration is viewed by the scientific community as dependent on the technique used and defined.

If further explanation is needed (and more detail): In the last set of experiments, we used 1% SSA for both plant related dho-B and mammalian Dh-OLF in an attempt to compare the two parent molecules (intact molecules) and their two genin products (i.e., sugars removed) after removing their single rhamnose from the parent molecules. In this set of experiments we used a specific antibody raised against dho-B. Note that the difference in the elution times indicate clearly the structural differences exists between the dho-B and mammalian counterpart Dh-OLF. Note the elution times of both parent molecules (Dho-B, 28 min) and DH-OLF, 26 min) and their genin components (Dho-B-genin, 20 min) and Dh-OLF-genin, 18 min) are different but within the chromatographic area originally described. Importantly, the HPLC elution times of the parent compounds are well within those reported previously (between 25 to 29 min) but now with greater resolution the two compounds do separate indicating structural differences between them.

Second, we will address the question raised about the inventors' own publication in 1997 and Renning. Because the inventors have shown definitively that the compounds are in fact structurally different, the claims are patentable. In the inventors' FASEB published abstract (Qazzaz et al., FASEB J. 1997;11(9): A1100), the inventors reported the presence of two plant-derived isomers (dho-A and dho-B) separated from a commercial preparation of dihydroouabain (dho). It should be noted that this publication dealt only with plant-derived (i.e., commercially available) dihydroouabain, it in no way mentions anything related to any mammalian-derived substance. It is also important to note that in that publication the inventors used absorbance to measure to elution of the two isomers (dho-B and dho-A). In other words, the absorbance of the plant-related isomers (dho-B and-A) is the only tool used to determine the elution time. In 1997, no antibodies had yet been raised against neither the dihydroouabain stock (containing both isomers) nor the dho-B isomer. Note that the range of the chromatographic elution time on the reverse phase HPLC column (injecting 3 mg dho) for the dho-B peak extends for 4 minutes (from 25 to 29 min) and thus the inventors reported 27.5 min as the mean of the range (top peak was at 27.5). Nevertheless, there is no connection between these findings and the present patent application other than that the inventors used similar chromatographic techniques to discover the subject mammalian Dh-OLF (dihydro-ouabain-like factor) compound a few years later.

Thus, the plant compounds (dho-B) and the mammalian derived compounds (Dh-OLF) migrated together during the initial preliminary experiments, but upon further experimentation (i.e., more sensitive technical refinement), they resolve. This resolution demonstrates by necessity an inherent structural difference existing between these compounds. Thus, the mammalian-derived compounds are patentable over the plant-derived compounds.